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Applicant(s) : POULSEN ET AL.  
Serial No. : 09/998,284  
Filing Date : NOVEMBER 30, 2001  
Title of Invention : COMPOSITION

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CLAIM OF PRIORITY

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Sir:

Applicants hereby claim priority under 35 U.S.C. §119 and for 120, from British application number GB9913050.2, a certified copy is enclosed.

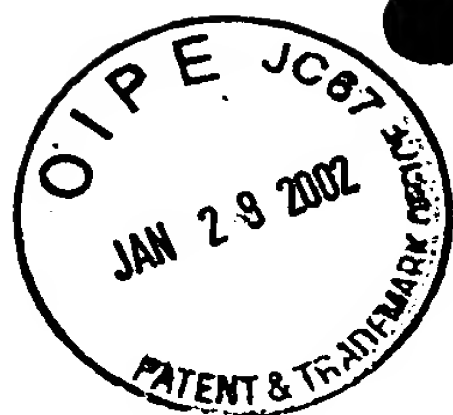
Acknowledgment of the claim of priority and of the receipt of said certified copy are respectfully requested.

Respectfully submitted,

FROMMER LAWRENCE & HAUG LLP

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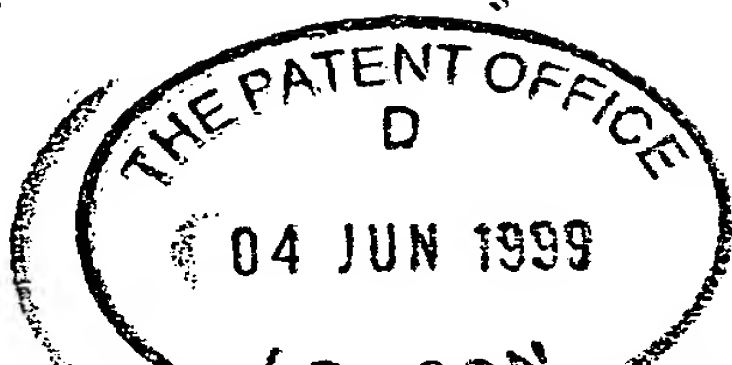
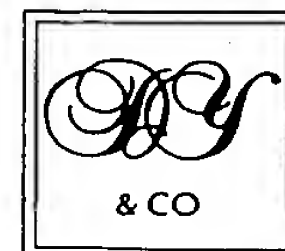
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Dated 19 December 2001





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Cardiff Road  
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1. Your reference P006441GB DAA

2. Patent application (The Patent Office) 9913050.2 04 JUN 1999

3. Full name, address and postcode of the or of each applicant (underline all surnames)  
Danisco A/S  
Langebrogade 1  
PO Box 17  
DK-1001 Copenhagen K  
Denmark

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

Denmark

5660873002

4. Title of the invention Anti-Fouling Composition

5. Name of your agent (if you have one) D YOUNG & CO  
"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode) 21 NEW FETTER LANE  
LONDON  
EC4A 1DA

Patents ADP number (if you have one)

59006

6. If you are declaring priority from one or more earlier patent applications, give the country and date of filing of the or each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of filing (day/month/year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and filing date of the earlier application	Number of earlier application	Date of filing (day/month/year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:  
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Description 14

Claims(s) 2

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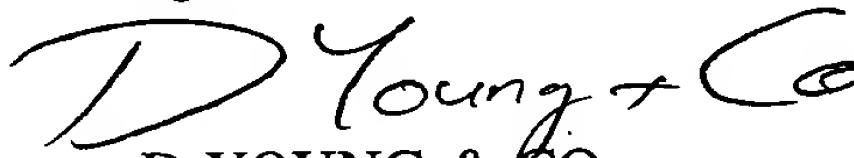
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I/We request the grant of a patent on the basis of this application.

Signature

Date

  
D YOUNG & CO  
Agents for the Applicants

4/6/1999

12. Name and daytime telephone number of the person to contact in the United Kingdom

D Alcock

01703 634816

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## Anti-Fouling Composition

The present invention relates to an anti-fouling composition. In particular, the present invention relates to an anti-fouling composition comprising an enzyme capable of  
5 producing a compound having an anti-fouling effect.

As discussed in US-A-5071479 biocides are required in many different environments, such as antifungal agents in house paints, fresh water algicides, and anti-fouling agents for marine structures exposed to sea water flora and fauna. As is known, mildew or  
10 fungus may grow on house paints and the like, and utilizes the paint medium as a nutrient, or in some cases, the underlying substrate, such as wood, as the nutrient. For obvious reasons, this may cause damage to the painted surface and/or a deterioration in the appearance of the painted surface. A biocide may be incorporated in the paint and when the mycelia and fruiting bodies of the fungi contact or penetrate the paint film and  
15 thus, through intimate contact with the biocide in the film, the fungi are destroyed. In cooling towers utilizing fresh water, slimes, mould and algae may develop if effective compounds for combating their growth are not present.

As discussed in US-A-5071479 the growth of marine organisms on the submerged parts  
20 of a ship's hull is a particular problem. Such growth increases the frictional resistance of the hull to passage through water, leading to increased fuel consumption and/or a reduction in the speed of the ship. Marine growths accumulate so rapidly that the remedy of cleaning and repainting as required in dry-dock is generally considered too expensive. An alternative which has been practiced with increasing efficiency over the  
25 years, is to limit the extent of fouling by applying to the hull a top coat paint incorporating anti-fouling agents. The anti-fouling agents are biocides which are freed from the surface of the paint over a period of time at a concentration lethal to marine organisms at the hull surface. The anti-fouling paint fails only when the concentration of biocide available at the paint surface falls below the lethal concentration and with  
30 modern paints up to two years of useful life is expected.

An extremely widely used biocide, particularly in marine anti-fouls, is tributyl tin

(TBT). However, there is a growing concern about the environmental effects caused by using such organic tin biocides at their present commercial levels as an anti-foulant active ingredient in coating compositions for aquatic (marine) applications. It has been shown that, due to the wide-spread use of tributyltin-type compounds in particular, at concentrations as high as 20 wt.% in paints for ship bottoms, the pollution of surrounding water due to leaching has reached such a level as to cause the degradation of mussel and shell organisms. These effects have been detected along the French-British coastline and a similar effect has been confirmed in U.S. and Far East waters. Under the most recent regulatory restrictions, with limited exceptions, pleasure boats up to 25 meters long are no longer permitted to use anti-foulant paint containing high levels of tributyltin compounds.

Research has shown that as long as the leaching rate of tin can be maintained at or below about  $4 \mu\text{g}/\text{cm}^2$  per day, aquatic life does not appear to be affected over the long term. However, it has also been found that to be effective for controlling marine algae, as well as higher developed marine organisms, from the painted surface of ship bottoms, a certain minimum leaching rate of tin of about 9 to  $16 \mu\text{g}/\text{cm}^2/\text{day}$  is required. Usually, this higher leaching rate is achieved with a concentration of tributyltin compound at about 15% to 20% by weight of paint.

20

In view of the effectiveness of TBT regulatory authorities have reluctantly agreed that as long as there is no satisfactory substitute for the anti-foulant organic tin active ingredients, larger ships, i.e., those above a length of 25 meters, are still permitted to use such compounds to minimize fouling. There is therefore a desire to provide alternative biocides to TBT based compounds.

25

US-A-4297137 discloses that the effects of an anti-fouling composition can be lengthened by moderating the release of the anti-fouling constituents. This document discloses anti-fouling paints comprising at least one substance toxic to marine organism uniformly incorporated into a discontinuous solid matrix which is insoluble in sea water and is dispersed in the paint. The matrix is at least partially formed from at least one substance which becomes soluble in sea water under the action of enzymes liberated by

30



the marine organisms to be inhibited and/or by the bacterial film in contact with the paint. Thus when marine organism become associated with the painted surface, the toxic substance is released and the organisms inhibited. Similar to prior art disclosures, the toxic substances envisaged by US-A-4297137 include only the well known copper and tin based compounds, such as TBT.

Abarzua *et al.*, Mar. Ecol. Prog. Ser., Vol. 123: 301-312, 1995 "*Biotechnological investigation for the prevention of biofouling. I. Biological and biochemical principles for the prevention of biofouling*" propose the extraction of biogenic agents having antibacterial, anti-algal, anti-protozoan and anti-macrofouling properties from algae and marine invertebrates. It is proposed that the structure of the extracted agents may be determined, subsequently synthesised and the synthesised agent used in the prevention of biofouling. No teaching of the extraction or of the synthesis is provided.

US-A-5747078 relates to food products. The document teaches that microbial contamination of food and feed, which can cause severe health problems, may be inhibited by an immobilised composition comprising a lactoperoxidase system which provides for the sustained release of hydrogen peroxide. The hydrogen peroxide is prepared by the reaction of an oxidoreductase with an oxidisable substrate. The hydrogen peroxide then reacts with thiocyanate, under catalysis by lactoperoxidase, to produce hypothiocyanate. The hypothiocyanate may then act as an anti-microbial agent. This document provides a background teaching of immobilised enzyme systems. The document is silent concerning anti-foulants or any micro-organism which results in fouling properties.

The present invention addresses the problem of the prior art

Aspects of the present invention are defined in the appended claims. These and other preferred aspects are discussed below.

In the present specification "foulants" referred to by the terms "anti-foul(s)", "anti-fouling", and "anti-foulants" include organisms which may reside and/or grow on the

surface to be treated with the present composition. The organisms include micro-organisms such as bacteria, fungi and protozoa, and algae. The organism may be marine organisms.

- 5 The composition of the present invention may comprise a precursor enzyme and a precursor substrate, wherein the precursor enzyme and the precursor substrate are selected such that the precursor substrate generates a substrate for the enzyme of the present invention by action of the precursor enzyme on the precursor substrate. This combination of precursor enzyme and precursor substrate is herein after referred to as  
10 "substrate generator".

Preferably, the compound is hydrogen peroxide.

- Preferably, the enzyme is selected from hexose oxidase, glucose oxidase, L amino acid  
15 oxidase, galactose oxidase, hexose oxidase,  $\beta$ -galactosidase, pyranose oxidase, cellulase, cellubiohydrolase, inulinase, amyloglucosidase, amylase, pectinolytic enzyme, xylanase, and mixtures thereof.

- Preferably, the substrate is selected from a sugars, including hexoses, preferably  
20 glucose, L amino acid, galactose, lactose, 2-deoxyglucose, pyranose, peptide, xylan, cellulose, inulin, starch, dextran, pectin, and mixtures thereof.

- In a highly preferred embodiment the enzyme/substrate combination is selected from glucose/hexose oxidase, glucose/glucose oxidase, L amino acid/L amino acid oxidase,  
25 galactose/galactose oxidase, lactose/ $\beta$ -galactosidase/hexose oxidase, lactose/ $\beta$ -galactosidase/glucose oxidase, 2-deoxyglucose/glucose oxidase, pyranose/pyranose oxidase, and mixtures thereof.

- In one aspect the anti-foulant compound is generated by action of the enzyme on the  
30 substrate which is present in the composition. Thus the anti-foulant compound is generated by a "one-step" process. In some cases the substrate may be prepared in situ. In these cases, the composition further comprises a precursor enzyme and a precursor

substrate wherein the precursor enzyme and the precursor substrate are selected such that the precursor enzyme generates the substrate. In this latter aspect the anti-foulant compound is generated by a "two-step" process

- 5 In the one-step process preferably the enzyme is selected from hexose oxidase, glucose oxidase, L amino acid oxidase, galactose oxidase, pyranose oxidase, and mixtures thereof.

In the one-step process preferably the substrate is selected from a hexose, preferably  
10 glucose, L amino acid, galactose, 2-deoxyglucose, pyranose, and mixtures thereof.

In the one-step process preferably the enzyme/substrate combination is selected from glucose/hexose oxidase, glucose/glucose oxidase, L amino acid/L amino acid oxidase, galactose/galactose oxidase, 2-deoxyglucose/glucose oxidase, pyranose/pyranose  
15 oxidase, and mixtures thereof.

In the two-step process preferably the enzyme is hexose oxidase.

In the two-step process preferably the substrate is glucose.  
20

In the two-step process preferably the precursor enzyme is amyloglucosidase.

In the two-step process preferably the precursor substrate is starch.

25 Thus in the two-step process preferably the precursor substrate/precursor enzyme/enzyme combination is starch/amyloglucosidase/hexose oxidase.

Preferably, the precursor substrate of the two-step process is selected from oligomers and polymers of substrates for oxidative enzymes, starch, lactose, cellulose, dextrose,  
30 peptide, inulin, and mixtures thereof.

Preferably, the precursor enzyme of the two-step process is selected from exo-acting

enzymes capable of degrading oligomeric or polymeric substrates to monomeric units, e.g.  $\beta$ -galactosidase, peptidase; amyloglucosidase, cellubiohydrolase, and mixtures thereof.

- 5 Preferably, the composition further comprises a binder to immobilise at least one of the constituents, preferably to immobilise the enzyme.

The compositions of the present invention may be formulated as coatings, lacquers, stains, enamels and the like, hereinafter referred to generically as "coating(s)".

10

Thus, in one aspect the present invention provides a coating consisting of a composition as defined above.

- 15 Preferably, the coating is formulated for treatment of a surface selected from outdoor wood work, external surface of a central heating system, and a hull of a marine vessel.

The coating may include a liquid vehicle (solvent) for dissolving or suspending the composition.

- 20 The liquid vehicle may be selected from any liquid which does not interfere with the activities of any essential components of the composition. In particular, the liquid vehicle should not interfere with the activity of the essential enzyme(s) and/or anti-foulant compound. Suitable liquid vehicles are disclosed in US-A-5071479 and include water and organic solvents including aliphatic hydrocarbons, aromatic hydrocarbons,  
25 such as xylene, toluene, mixtures of aliphatic and aromatic hydrocarbons having boiling points between 100 and 320°C, preferably between 150 and 230°C; high aromatic petroleum distillates, e.g., solvent naptha, distilled tar oil and mixtures thereof; alcohols such as butanol, octanol and glycols; vegetable and mineral oils; ketones such as acetone; petroleum fractions such as mineral spirits and kerosene, chlorinated  
30 hydrocarbons, glycol esters, glycol ester ethers, derivatives and mixtures thereof.

The liquid vehicle may contain at least one polar solvent, such as water, in admixture

with an oily or oil-like low-volatility organic solvent, such as the mixture of aromatic and aliphatic solvents found in white spirits, also commonly called mineral spirits.

The vehicle may typically contain at least one of a diluent, an emulsifier, a wetting agent, a dispersing agent or other surface active agent. Examples of suitable emulsifiers are disclosed in US-A-5071479 and include nonylphenol-ethylene oxide ethers, polyoxyethylene sorbitol esters or polyoxyethylene sorbitan esters of fatty acids, derivatives and mixtures thereof.

Any suitable surface coating material may be incorporated in the composition and/or coating of the present invention. Examples of trade-recognized coating materials are polyvinyl chloride resins in a solvent based system, chlorinated rubbers in a solvent based system, acrylic resins and methacrylate resins in solvent based or aqueous systems, vinyl chloride-vinyl acetate copolymer systems as aqueous dispersions or solvent based systems, butadiene copolymers such as butadiene-styrene rubbers, butadiene-acrylonitrile rubbers, and butadiene-styrene-acrylonitrile rubbers, drying oils such as linseed oil, alkyd resins, asphalt, epoxy resins, urethane resins, polyester resins, phenolic resins, derivatives and mixtures thereof.

The composition and/or coating of the present invention may contain pigments selected from inorganic pigments, such as titanium dioxide, ferric oxide, silica, talc, or china clay, organic pigments such as carbon black or dyes insoluble in sea water, derivatives and mixtures thereof.

The composition and/or coating of the present invention may contain materials such as rosin to provide controlled release of the anti-foulant compound, rosin being to a very slight extent soluble in sea water.

The composition and/or coating of the present invention may contain plasticisers, rheology characteristic modifiers, other conventional ingredients and mixtures thereof.

The composition and/or coating of the present invention, particularly the coating, further

comprise with an adjuvant conventionally employed in compositions used for protecting materials exposed to an aquatic environment. These adjuvants may be selected from additional fungicides, auxiliary solvents, processing additives such as defoamers, fixatives, plasticisers, UV-stabilizers or stability enhancers, water soluble or water insoluble dyes, color pigments, siccatives, corrosion inhibitors, thickeners or anti-settlement agents such as carboxymethyl cellulose, polyacrylic acid or polymethacrylic acid, anti-skinning agents, derivatives and mixtures thereof.

The additional fungicide(s) used in the composition and/or coating of the present invention is preferably soluble in the liquid vehicle.

In one aspect the present invention provides a marine anti-foulant consisting of a composition as defined above.

Preferably, the anti-foulant is self-polishable.

In one aspect of the present invention, the substrate or substrate generator and/or the enzyme are encapsulated. Preferably, the substrate/substrate generator and/or enzyme are encapsulated by a semi-permeable membrane.

20

The substrate/substrate generator and enzyme may be encapsulated individually independently of each other or may be encapsulated together. In the former embodiment, the substrate/substrate generator or enzyme may be activated by the foulant. For example, the encapsulating material may be selected such that on contact with a foulant, the substrate/substrate generator or enzyme may be released to contact the other of the substrate/substrate generator or enzyme. In this way, a composition may be provided which only provides an anti-foulant compound or increases provision of an anti-foulant compound when contacted with a foulant.

The composition of the present invention can be provided as a ready-for-use product or as a concentrate. The ready-for-use product may be in the form of an aqueous solution, aqueous dispersion, oil solution, oil dispersion, emulsion, or an aerosol preparation.

The concentrate can be used, for example, as an additive for coating, or can be diluted prior to use with additional solvents or suspending agents.

5 An aerosol preparation according to the invention may be obtained in the usual manner by incorporating the composition of the present invention comprising or dissolved or suspended in, a suitable solvent, in a volatile liquid suitable for use as a propellant, for example the mixture of chlorine and fluorine derivatives of methane and ethane commercially available under the trademark "Freon", or compressed air.

10 As discussed in US-A-5071479 the composition and/or coating of the present invention may include additional ingredients known to be useful in preservatives and/or coatings. Such ingredients include fixatives such as carboxymethylcellulose, polyvinyl alcohol, paraffin, co-solvents, such as ethylglycol acetate and methoxypropyl acetate, plasticisers such as benzoic acid esters and phthlates, e.g., dibutyl phthalate, dioctyl phthalate and  
15 didodecyl phthalate, derivatives and mixtures thereof. Optionally dyes, color pigments, corrosion inhibitors, chemical stabilizers or siccatives (dryers) such as cobalt octate and cobalt naphthenate, may also be included depending on specific applications.

The composition and/or coating of the present invention can be applied by any of the  
20 techniques known in the art including brushing, spraying, roll coating, dipping and combinations thereof.

Compositions of the present invention can be prepared simply by mixing the various ingredients at a temperature at which they are not adversely affected. Preparation  
25 conditions are not critical. Equipment and methods conventionally employed in the manufacture of coating and similar compositions can be advantageously employed.

The invention will now be described, by way of example only, in the following Examples.

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#### **EXAMPLE**



The anti-fouling effect of an anti-fouling composition of the present invention is tested according to the following examples. These Example show the effectiveness of the present composition at preventing fouling. The Examples also provide for the optimisation of the anti-fouling properties of the present composition.

5

#### **Set Up Of A Test System For An Anti-fouling Composition**

0.5 to 5 ml. samples of lake or sea water were collected in test tubes from the lake Brabrandssøen near Aarhus, Denmark, and from the Baltic sea off Aarhus. On the day of  
10 collection of the water samples the anti-fouling composition to be tested is added to the test tubes and they are sealed with Parafilm™.

The test tubes are incubated at room temperature in proximity to a window to allow daylight to fall thereon. Fouling is monitored visually for up to 4 weeks based on the  
15 appearance of a microbial growth layer on the walls of the test tube. For comparison a test tube with 0.1% of sodium azide and a test tube without anti-fouling composition are used as positive and negative controls, respectively.

These test tubes are rated 1 and 5, respectively, on a scale of 1 to 5 for highly efficient  
20 to no anti-fouling activity, respectively.

#### **EXAMPLE 1 - Preparation Of An Anti-fouling Composition ("One-Step")**

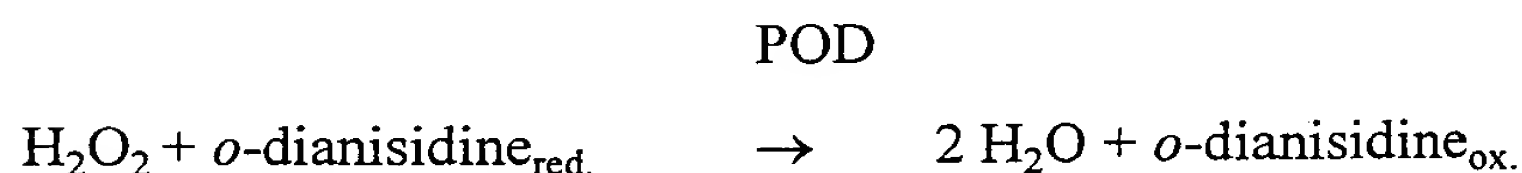
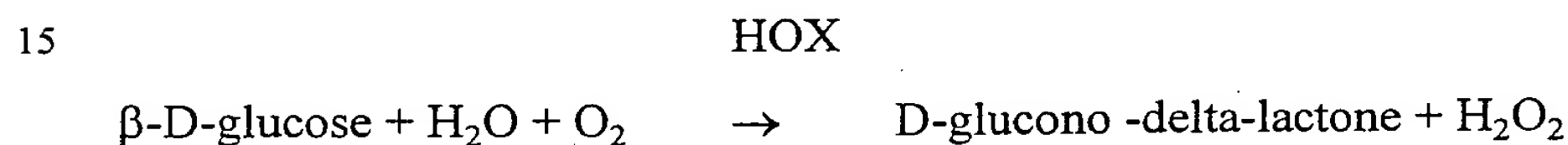
Soluble or immobilised hexose oxidase or another hydrogen peroxide generating  
25 enzyme such as glucose oxidase is tested as a anti-foulant compound generating enzyme in an anti-fouling composition. The hexose oxidase is immobilised. This may be achieved by binding to an anion exchanger, Q Sepharose FF™ (available from Pharmacia) using 20 mM triethanolamine buffer, pH 7.3. Alternatively, hexose oxidase or alternative hydrogen peroxide generating enzymes is covalently linked to a suitable  
30 carrier such as epoxy activated Sepharose™ (Pharmacia, Sweden), carbodiimide activated agarose (Bio-Rad, USA). Other conventional procedures known in the art for immobilisation may also be utilised



The range of concentrations used is 0.0001 to 1000 U of hexose oxidase activity/hydrogen peroxide generating enzyme per ml of anti-fouling composition. One unit of enzyme activity is defined as the amount of enzyme which produces 1  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2$  per min at 25 °C.

To ascertain its suitability for use in the present invention the activity of the enzyme may be assayed as follows. Hexose oxidase (HOX) activity is measured in accordance with the following procedure.

The HOX assay is based on the measurement of hydrogen peroxide generated in the oxidation of glucose. The hydrogen peroxide oxidises *o*-dianisidine in the presence of peroxidase to form a dye.



20

#### *Reagents*

1. 100 mM phosphate buffer, pH 6.3
2. 100 mM D-glucose (SIGMA, G-8270) in 100 mM phosphate buffer, pH 6.3
3. *o*-Dianisidine (SIGMA, D-3252), 3.0 mg/ml in distilled water
- 25 4. Peroxidase (SIGMA, P-8125), 0.10 mg/ml in 100 mM phosphate buffer, pH 6.3

#### *Assay*

- 120  $\mu\text{l}$  reagent 1
- 150  $\mu\text{l}$  reagent 2
- 30 10  $\mu\text{l}$  reagent 3
- 10  $\mu\text{l}$  reagent 4 and
- 10  $\mu\text{l}$  enzyme solution

The assay is performed in a microtiter plate. The reaction is initiated by the addition of enzyme solution. The mixture is incubated at 25°C for 15 min with shaking. The blank run contains all the components with water instead of enzyme solution. The formation of the dye is measured in a microtiter plate reader at 405 nm. The linearity of the reaction can be checked by using a kinetics programme on the microplate reader.

A hydrogen peroxide standard curve can be constructed by using varying concentrations of fresh  $\text{H}_2\text{O}_2$  (MERCK, 1.07209). One unit of enzyme activity is defined as the amount of enzyme which produces 1  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2$  per min at 25 °C.

### **EXAMPLE 2 - Preparation Of An Anti-fouling Composition ("Two-Step")**

Glucose and galactose in concentrations of 0.01 to 100  $\mu\text{g}$  per ml of anti-fouling composition are tested as a precursor substrate and a precursor enzyme to generate a substrate for hexose oxidase in the systems described in Example 1. In order to provide a continuous substrate generating system, starch, preferably intact starch granules from wheat, maize or potato, in a concentration of from 0.01 ng to 100  $\mu\text{g}$  per ml of anti-fouling composition, are used together with amyloglucosidase (GRINDAMYL™ AG 1500 Bakery Enzyme from Danisco Ingredients or another commercial amyloglucosidase product). The components are present in concentrations providing from 0.000001 to 10 AGU per ml of anti-fouling composition.

1 AGU is defined as the amyloglucosidase activity which releases 1 mmol of glucose per minute from maltose (0.5% w/v) in 50 mM sodium acetate, pH 5.0 (adjusted with concentrated acetic acid) at 40°C. The assay is stopped by transferring 200 ml of assay mix to 100 ml of 0.1 M hydrochloric acid chloride and the amount of glucose released is measured using glucose dehydrogenase reagent (Merck no. 12193) or another glucose detection system.

30

### **EXAMPLE 3 - Model System For Coating**

Dialysis tubing containing an anti-fouling composition is used as a model system for a coating to prevent fouling on the surface of a coated material.

5 An anti-fouling composition within the dialysis tubing is used to generate an concentration of hydrogen peroxide on the surface of the dialysis tubing effective to prevent fouling. The dialysis tubing used has a cut off value of about 10000 Da. The dialysis tubing is either dialysis tubing or a dialysis cassette (such as Slide-A-Lyzer™ available from Pierce; IL, USA).

10 The dialysis tubing is immersed in a glass beaker with 1 to 5 litre of lake or sea water collected as described above. The glass beaker is stirred slowly with a magnetic stirrer and incubated at room temperature in proximity to a window to allow daylight to fall thereon. Fouling on the dialysis tubing is monitored visually for up to 4 weeks based on the appearance of a microbial growth layer on the dialysis tubing and rated on a scale of  
15 1 to 5 as described above. As negative control a dialysis tube containing tap water is used.

Optionally, catalase immobilised onto nitrocellulose membrane pieces, which have subsequently been blocked with 0.1% Tween 20, are added to the lake or sea water in  
20 order to remove hydrogen peroxide from the water surrounding the dialysis tubing. The concentration of catalase used is in the range of 0.000001 to 100 CU, where 1 CU is defined as the catalase activity degrading 1 mmol of hydrogen peroxide per minute at 30°C in 50 mM sodium phosphate buffer, pH 7.0, as described for catalase in the Sigma catalogue: Biochemicals Organic Compounds for Research and Diagnostic Reagents,  
25 Sigma Chemical Company 1995, page 221.

The compositions of the present invention are effective at preventing fouling.

#### **EXAMPLE 4 -Testing Of Coating**

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Commercial marine anti-fouling coating material without added anti-fouling biocide is used. Anti-fouling compositions according to the present invention are mixed into the

coating material and applied to the surface of metal, glass and plastic plates according to the instructions of the manufacturer of the coating material.

Coated plates are immersed into water in a lake or in sea water. Fouling on the plates is  
5 monitored visually for up to 2 years based on the appearance of a microbial growth layer on the plates and rated on a scale of 1 to 5 as described above. As negative control a coating without anti-fouling composition is used.

The compositions of the present invention are effective at preventing fouling.

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All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the  
15 scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry or related fields are intended to be  
20 within the scope of the following claims.

CLAIMS

1. An anti-fouling composition comprising
  - (i) a surface coating material;
  - 5 (ii) an enzyme; and
  - (iii) (a) a substrate for the enzyme; and/or  
(b) a precursor enzyme and a precursor substrate, wherein the precursor enzyme and the precursor substrate are selected such that the precursor substrate generates a substrate for the enzyme by action of the precursor enzyme on the  
10 precursor substrate;wherein the enzyme and the substrate are selected such that the substrate generates an anti-foulant compound by action of the enzyme on the substrate.
2. A composition according to claim 1 wherein the anti-foulant compound is  
15 hydrogen peroxide.
3. A composition according to claim 1 or 2 wherein the enzyme is hexose oxidase.
4. A composition according to claim 1, 2 or 3 wherein the substrate is a sugar.  
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5. A composition according to claim 4 wherein the sugar is glucose.
6. A composition according to any one of the preceding claims wherein the composition comprises a precursor enzyme and a precursor substrate, wherein the  
25 precursor enzyme and the precursor substrate are selected such that the precursor substrate generates a substrate for the enzyme by action of the precursor enzyme on the precursor substrate.
7. A composition according to claim 6 wherein the precursor enzyme is  
30 amyloglucosidase.
8. A composition according to claim 6 or 7 wherein the precursor substrate is

starch.

9. A composition according to any one of the preceding claims wherein the composition further comprises a binder to immobilise at least one of the constituents of  
5 the composition, preferably to immobilise the enzyme.

10. A coating consisting of a composition according to any one of the preceding claims.

10 11. A coating according to claim 10 formulated for treatment of a surface selected from outdoor wood work, external surface of a central heating system, and a hull of a marine vessel.

12. A marine anti-foul consisting of a composition according to any one of the  
15 preceding claims.

13. A marine anti-foul according to claim 12 wherein the anti-foulant is self-polishable.

20 14. A composition as substantially hereinbefore described with reference to the Examples.

15. A coating as substantially hereinbefore described with reference to the Examples.

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16. A marine anti-foul as substantially hereinbefore described with reference to the Examples.

**ABSTRACT**

There is provided an anti-fouling composition comprising (i) a surface coating material;  
5 (ii) an enzyme; and (iii) (a) a substrate for the enzyme; and/or (b) a precursor enzyme  
and a precursor substrate, wherein the precursor enzyme and the precursor substrate are  
selected such that the precursor substrate generates a substrate for the enzyme by action  
of the precursor enzyme on the precursor substrate; wherein the enzyme and the  
substrate are selected such that the substrate generates an anti-foulant compound by  
10 action of the enzyme on the substrate.

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